PB83-157081

Behavioral Alterations Due to Diesel Exhaust Exposure

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1981

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1. REPORT NO.	2.	3
EPA-600/J-81-606	JOURNAL ARTICLE	PB83-157081

4. TITLE AND SUBTITLE	5. REPORT DATE
JOURNAL ARTICLE: BEHAVIORAL ALTERATIONS DUE TO DIESEL EXHAUST EXPOSURE	1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R. Dana Laurie, William K. Boyes, & Thomas Wessendarp	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS TOTB/TMD/HERL/USEPA 26 West St. Clair Street Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. A9XAIC 11. CONTRACT/GRANT NO. In-house
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Environmental International, Vol. 5, pp. 359-361, 1981

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	unclassified	6
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE

Environment International, Vol. 5, pp. 357–361, 1981
Printed in the USA.

0160-4120/81/040357-05$02.00/0

EPA-600/J-81-606
JOURNAL ARTICLE

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BEHAVIORAL ALTERATIONS DUE TO DIESEL EXHAUST EXPOSURE

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Several experiments examining the effects of diesel exhaust on the behavior of rats are reported. Animals were exposed either as adults or neonates. The spontaneous locomotor activity (SLA), measured in standard running wheel cages, of adult rats exposed for 8 h/day, 7 days/week was significantly less than that of controls. Experiments involving diesel exhaust exposure to neonatal rats indicated that adult rats, exposed to diesel exhaust during their neonatal lives, were significantly less active as measured by SLA. Adult rats, exposed to 20 h diesel per day as neonates, were placed in skinner boxes after the SLA experiment described above had been completed. The exhaust exposed animals showed significantly decreased acquisition of a food reinforced bar pressing task. All animals that learned this task extinguished at the same rate. The results of the neonatal diesel exhaust experiments support the hypothesis that diesel exhaust exposure during development of an organism can lead to behavioral differences in adulthood.

Introduction

These experiments were conducted as part of an overall EPA program studying the health effects of diesel exhaust. The objective of this section of the program was to measure the behavioral consequences of diesel exhaust exposure to adult and neonatal animals. Behavior was used as a measureable end point because it can be a general indicator of the health status of an animal.

It was previously reported (Laurie *et al.*, 1978) that exposure of rats to approximately 6 mg/m^3 diesel exhaust for 20 h/day, 7 days/week significantly reduced two types of spontaneous activity: (1) adult spontaneous locomotor activity, and (2) neonatal pivoting. The work described in this paper is a continuation of the study of the effects of diesel exhaust exposure on behavior.

Two sets of experiments are reported in this paper. The first set examines the hypothesis that the depression in running wheel behavior previously observed in adult rats exposed to diesel exhaust for 20 h/day would be less severe in adults exposed to only 8 h/day. The second set of experiments examine the hypothesis that neonatal exposure to diesel exhaust will produce alterations in adult behavior. In conjunction with one of the neonatal experiments, electrophysiological recordings were collected from litter mates of animals used in the behavioral experiments.

Methods

Experiment involving exposure of adult rats

Exposure. The diesel exhaust group was exposed for 8 h/day (6:30 am to 2:30 pm), 7 days/week, to diesel exhaust generated by a Nissan CN6-33 engine coupled to a Chrysler Torque-flite automatic transmission, Model A-727. The raw exhaust was diluted with filtered air in a ratio of approximately 1:20 to achieve a particulate matter concentration of 5.97 ± 0.17 mg/m^3 during the 16 weeks of exposure [weeks 10 through 25 of the EPA program reported by Hinners *et al.* (1979)]. Concentrations of the measured components of the diesel exhaust are listed in Table 1. The control group was exposed to filtered air only.

Spontaneous locomotor activity. Spontaneous locomotor activity (SLA) measurements were obtained by placing adult Charles River Sprague Dawley rats (45 days post parturition) into standard, 14 cm running wheels. Two weeks of baseline data were obtained for 50 rats, housed in a controlled environment. In an attempt to reduce the

Table 1. Average levels (± S.E.M.) of diesel exhaust components during adult exposures.

Component	Control Air	Diesel Exhaust
CO	1.86 ± 0.06	19.20 ± 0.35
CO_2(%)	0.05 ± 0.00	0.28 ± 0.01
Hydrocarbons	3.22 ± 0.08	7.29 ± 0.11
NO	0.08 ± 0.01	11.14 ± 0.43
NO_2	0.03 ± 0.00	2.51 ± 0.10
Particulate (mg/m^3)	0.01	5.97 ± 0.17
SO_2	0.46 ± 0.02	1.82 ± 0.07

amount of variability in the data, the very high and very low runners were excluded. Of the original 50, 26 rats were pair matched for SLA levels, the group size being limited by the size of available exposure chambers. One rat from each pair was randomly assigned to either the control air or diesel exhaust group. The remaining rat from each pairing was placed in the opposite exposure situation.

The rats remained in their respective chambers for 16 weeks. Rats received standard laboratory chow and tap water *ad libitum*. Each week body weights were taken and the food and water weighed and changed. The chambers were hosed daily and scrubbed with "scum remover" weekly. The reading from the mechanical wheel revolution counter was recorded daily.

Experiments involving exposure of neonatal rats

Exposure. There were three neonatal exposures to diesel exhaust. Table 2 lists the three exposures and indicates the length of exposure per day, the age of the neonates during their exposure, the number of litters and the number of animals per group. The concentrations of the various exhaust components were similar to those during the adult exposure experiments.

Animal care. For each of the neonatal experiments, timed pregnant Sprague Dawley rats were ordered from the animal supplier. The day after parturition each litter was culled to eight pups containing either four, five or six males. The litters were then pair matched for dam weight, litter size, male to female ratio, pup weight, length of gestation and time of day parturition began. One member of each pair was randomly assigned to either the control air or diesel exhaust exposure condition and the remaining litter received the other treatment. After the exposure schedules depicted in Table 1 were concluded, the litters were removed from the chambers, transferred to another building and reared in a standard laboratory environment.

Table 2. Exposure conditions for neonatal rat experiments.

Exposure Situation	Hours of Exposure per day	Age of Neonates During Exposure (days post paturition)	No. of Litters per Group	No. of Animals per Group
1	20	1–17	5	12
2	8	1–21	12	15
3	8	1–28 or 42	14	28

Spontaneous locomotor activity. At the age of six or seven weeks post parturition, the neonatally exposed animals were placed into standard running wheels and measurement of SLA began. Readings from the wheel revolution counters were recorded daily. Body weights and food and water consumption were monitored weekly.

Bar pressing task. When the rats exposed to 20 h diesel exhaust per day as neonates (Exposure Situation 1 of Table 1) were 15 months of age, an attempt was made to train them to press a bar for food pellet reward. Prior to the onset of training the rats were placed on a food restricted diet until they achieved 80% of their previous body weight. Upon reaching the 80% criterion the animals were placed in an enclosed, ventiliated 30×30 cm Skinner Box containing a bar press mechanism adjacent to a food pellet cup on one wall of the chamber. Standard blind psychological shaping procedures were used to train the rats to bar press. Following acquisition of the task, performance was maintained on a continuous reinforcement schedule (CRF). After 42 days of CRF, the task was switched to an extinction condition in which the rats were placed in the boxes but did not receive food pellets for bar presses.

Results

Adult rat exposures

The SLA data obtained from rats exposed to diesel exhaust as adults are represented in Figs. 1 and 2. Both groups show a typical inverted U shaped pattern of running wheel behavior over time. During the 2 week baseline period (Fig. 1), the control and experimental groups are similar because of pair matching. Week 3 SLA was much lower than week 2, probably as a result of the movement of the animals into the exposure chambers. As the experiment progressed, both groups showed increased SLA, with the control group tending

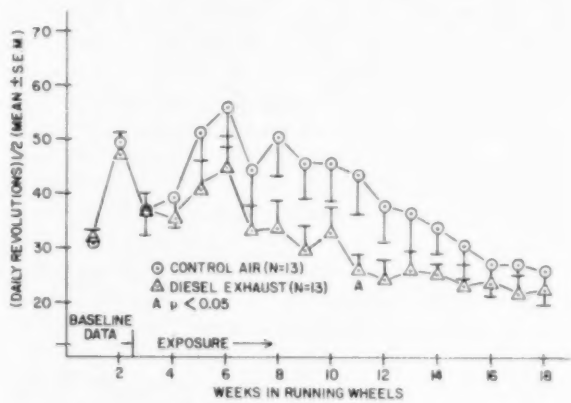

Fig. 1. Spontaneous locomotor activity (SLA) of rats exposed to either control air or diesel exhaust [8 h/day (0630 to 1430) SLA measurements began 6 weeks post parturition (pp)]. Exposure began 8 weeks pp.

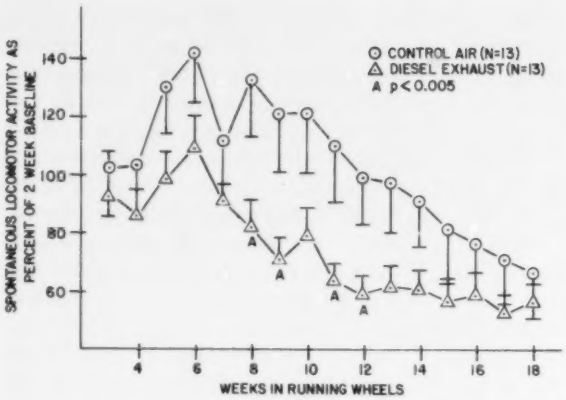

Fig. 2. Spontaneous locomotor activity (SLA) (expressed as percent of 2 weeks baseline) of rats exposed to either control air or diesel exhaust [8 h/day (0630 to 1430)]. SLA measurements began 6 weeks post parturition (pp). Exposure began 8 weeks pp.

to be more active than the pair matched exposed group. However, the only period in which the group differences reached statistical significance was week 11 of the experiment. The data were also analyzed in an alternate method by expressing each animal's SLA as a percentage of the two week baseline period. In this analysis, each animal serves as his own control, and the variability of the data is reduced. As depicted in Fig. 2, when SLA is expressed as a percentage of baseline, the diesel group is significantly less active than the control group during 4 weeks of the experiment (weeks 8, 9, 11, and 12). Significant decreases in food and water consumption for the exposed group correlated with decreased activity trends. Body weights were not significantly different.

Neonatal rat exposures

SLA of rats exposed for 20 *h/day*. Figure 3 consists of the adult SLA data from rats exposed to diesel exhaust for 20 h/day as neonates. Both groups show a typical pattern of running wheel behavior over time, however the exhaust exposed animals were significantly less active than control animals from week 5 to the end of the study.

SLA of rats exposed for 8 *h/day*. The SLA data from the first 8 h/day neonatal exposure experiment (Table 2, exposure situation 2) was highly abnormal in character. Neither control nor exposed animals demonstrated a typical inverted U shaped function over time. Activity levels for both groups remained at a low level throughout the experiment, with control animals running only 17% as much as the controls in the previous experiment. The SLA values for control and exposed animals for this experiment were not significantly different. An adequate explanation for the low SLA values demonstrated by both groups in the first 8 h/day experiment has not been reached.

A second experiment exposing neonates to 8 h diesel exhaust per day was conducted because of the abnormal

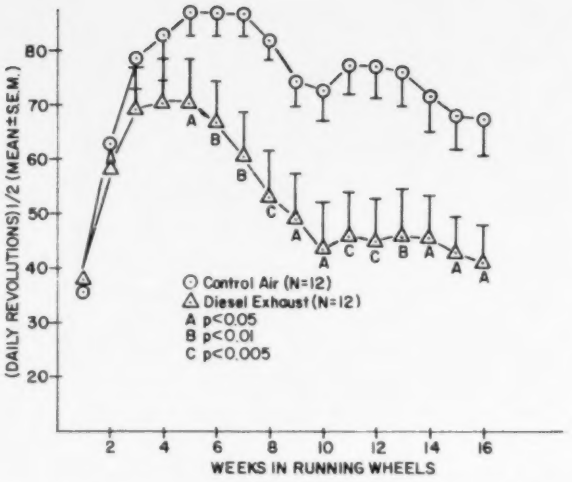

Fig. 3. Spontaneous locomotor activity (SLA) of rats exposed to either control air or diesel exhaust [20 h/day, days 2 through 18 post parturition (pp)]. SLA measurements began 7 weeks pp.

data from the first 8 h/day experiment. The second experiment contained 2 diesel groups, exposed from days 1 to 28 or 1 to 45 post parturition, and 2 corresponding control groups (Table 2, Exposure Situation 3). There were no significant differences within either exposure condition. Therefore, the data from the two exposure and the two control groups were pooled to achieve the groups represented in Fig. 4. Both control and exposed groups showed a typical inverted U shaped SLA function over time, with the control group demonstrating a normal level of SLA. The neonatally exposed group was significantly less active than controls during each week of the experiment from weeks 5 to 13, with the exception of weeks 7 and 11.

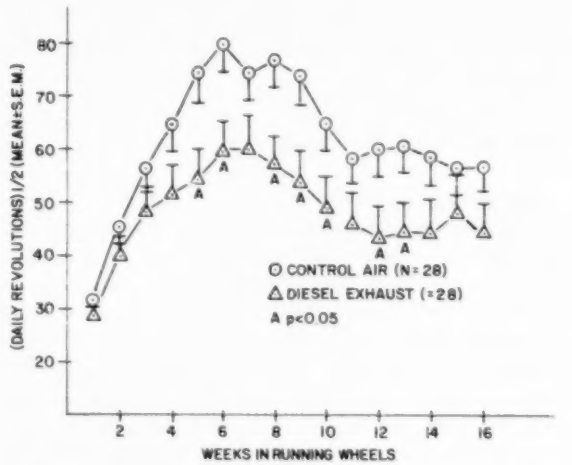

Fig. 4. Spontaneous locomotor activity (SLA) of rats exposed to either control air or diesel exhaust [8 h/day, days 1 through 28 or 45 post parturition (pp)]. SLA measurements began 6 weeks pp.

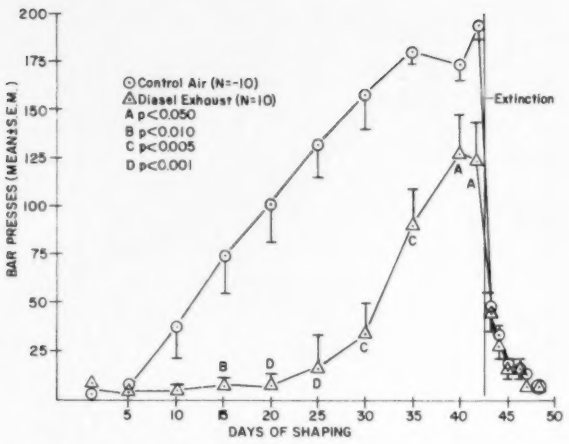

Fig. 5. Bar presses for food reward of rats exposed to either control air or diesel exhaust (20 h/day, days 2 to 18 post parturition). Training began at 15 months of age.

Bar pressing acquisition for rats exposed for 20 h/day. The results of the bar pressing acquisition task are presented in Fig. 5. After a few experimental sessions, animals in the control group began to press the bar. With continued experience in the task, the control animals showed a steady increase in the number of bar presses registered per day. In contrast, the diesel exposed group acquired the task only after extensive training. After 25 days of shaping, only one of the 10 diesel animals had learned to press the bar. With continued training most of the diesel animals did begin to bar press. However, after 42 days of shaping 2 of the 10 had not acquired the task. From the 13th to the 42nd day of the experiment, the diesel group registered significantly fewer bar presses per day than the control group. When switched to extinction conditions on day 43, both groups showed a rapid decline in the number of bar presses registered per day. There were no statistically significant differences between exposed and control groups during extinction.

Discussion and Conclusions

Adult exposure

Exposure to diluted diesel exhaust lowered the SLA of adult rats. In the previous study (Laurie *et al.*, 1978), rats exposed to 20 h/day diesel exhaust (8:00 pm to 4:00 pm the next day) failed to show a typical inverted U shaped running pattern over time, and were significantly less active than controls each week of the 6 week exposure period and one week afterward. In contrast, rats exposed to diesel exhaust 8 h/day did show a typical increase and subsequent decrease in SLA over time, and were significantly less active than controls for 1 or 4 weeks of the experiment, depending on the data analysis. The greater effect of the 20 h/day exposure could be due to two factors. Not only were the 20 h/day animals exposed to a greater amount of diesel exhaust per day, but the exposure occurred during the night time which is the period of highest activity for the rat. In contrast, the 8 h/day exposure occurred during a period of the day during which rats are normally quiescent. It follows that the greater depression of SLA found in the 20 h/day group could be due to either a greater dosage of exhaust, or to exposure during the period of high SLA activity, or both. The fact that SLA depression was found in the 8 h/day group who were not being exposed to exhaust during the period of the night when most SLA occurs suggests that the depression must have been caused by a residual effect of the exhaust. The residual effect could have been either due to some components of the exhaust remaining in the chamber after the exhaust was turned off, or due to some effect on the animals themselves.

A comparison can be made of adult SLA data obtained from diesel exhaust experiments with similar data obtained from automobile exhaust experiments (Cooper *et al.*, 1977). For example, the 20 h per day exposure to an 18:1 dilution of diesel exhaust resulted in SLA that was only 7% of control level. Experiments involving 24 h/day exposure to an 11:1 dilution of untreated or catalytically treated automobile exhaust reduced the exposed rat's SLA to only 60% of the control level. Although comparisons of this sort must be made with caution considering interexperiment variability, these experiments provide evidence suggesting that diesel exhaust is more potent at reducing SLA than automobile exhaust.

Based on reports in the literature (Laurie *et al.*, 1978; Cooper *et al.*, 1977; Cooper *et al.*, 1979; Lewkowski, 1978; Lewkowski *et al.*, 1979), several individual components of the exhaust, including CO, H_2SO_4, NO, NO_2, and SO_2 can be ruled out as causative factors in the reduction of SLA in exposed adults. None of these compounds suppresses SLA to an extent comparable to diesel exhaust. This leads to the conclusion that the components of the exhaust that are responsible for the observed effects are either the hydrocarbons, particulate matter or some combination of the single constituents. In order to discern whether the effects are due to the gaseous components or the particulate matter, it would be beneficial to examine the effects of exposure to whole exhaust and whole exhaust minus the particulate matter as compared to control air.

Neonatal exposures

Two of the neonatal experiments (Table 2, Exposure Situations 1 and 3) suggest that there may be permanent sequalae associated with diesel exhaust exposure during the neonatal life of the rat. As adults, the two groups of animals exposed to diesel exhaust during development were significantly less active than their respective control groups. In addition, it can be seen that the dif-

ferences between the control and exposed groups were greater for the 20 h/day exposure versus the 8 h/day exposure. This suggests a dose–response relationship that is qualitatively similar to the adult effect.

The results from Exposure Situation 2 of Table 2 are difficult to interpret because of the unusually low activity levels displayed by both control and exposure groups. Neither group demonstrated signs of illness or other possible reasons for the failure to acquire normal running wheel behavior patterns. Whatever the case may be, this particular experiment cannot be taken as positive or negative evidence of the health hazards of diesel exhaust exposure.

The difficulty the diesel exhaust animals (Exposure Situation 1 of Table 2) had in acquiring the bar pressing task is further evidence that exposure to diesel exhaust during development may produce long lasting differences in behavior. The nature of the behavioral deficit is not clear. It cannot be determined from this experiment whether the differences in task acquisition are due to a learning deficit or to some other reason (e.g., motivational or arousal differences).

At this time, it is impossible to attribute the effects of the neonatal exposure to any particular constituent(s) of diesel exhaust. There is little comparable research. Components such as CO, hydrocarbons and particulate matter may play an important role in causing the observed behavioral differences.

Future research

To date, risk assessment for diesel exhaust exposure has concentrated on the possible oncogenetic properties of the exhaust. The research reported here indicates that there are long lasting effects attributable to diesel exhaust exposure in addition to carcinogenicity. In order to fully assess the risk of exposure, all potential health problems must be investigated. Many projects could be proposed that would contribute to the understanding of the behavioral consequences of exposure to diesel exhaust: (1) dose–response relationships should be examined in depth; (2) various constituents of diesel exhaust should be examined to determine their relative contribution to the observed toxic effects; (3) effort needs to be expended in development of human risk assessment models based on behavioral data; (4) the site of the lesion(s) should be localized; (5) species differences need to be evaluated; and (6) comparisons between diesel and gasoline engines need to be made, especially relative to the neonatal effects.

Acknowledgements—The author is grateful to Julius Williams for his technical assistance and Verna Tilford and Debbie Dean for their clerical assistance. The views expressed in this paper are those of the authors and do not necessarily represent those of the U.S. Environmental Protection Agency.

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